DOMINANT LETHALITY AND CORRELATED CHROMOSOME EFFECTS IN HABROBRACON EGGS X-RAYED IN DIPLOTENE AND IN LATE METAPHASE I [1]

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Introduction

If oviposition is prevented in well-fed females of the parasitic wasp Habrobracon by witholding them from their host they continue to produce mature eggs until the egg sacs are filled. These stored eggs may number as many as twenty per female and are in late metaphase of the first meiotic division (metaphase I). Their retention in this stage for four days has no effect on their hatchability which is 96 per cent in the wild type stock used for the experiments herein described.

When unmated females with stored eggs are x-rayed and allowed to oviposit at 30° C. all eggs laid during the first six hours after treatment will have been irradiated in late metaphase I. Eggs laid during the seventh and eighth hours after treatment consist of a variable mixture treated in metaphase I and in late diplotene (including all eggs in diakinesis) and are, therefore, of no use in the present study. Eggs laid during the ninth to twelfth hours after treatment will have been post-synaptic with their diffuse chromosomes in a relatively quiescent condition when irradiated. These are designated as late and early diplotene.

An advantage in the use of these eggs for the detection of injuries lies in the fact that they develop parthenogenetically if unfertilized and so indicate directly the effects of treatment on a haploid set of chromosomes. Disadvantages are the large number ($n = 10$) and small size (less than 1 μ in diameter) of their chromosomes. The details of oogenesis appear to be orthodox and so the results should be universally applicable to forms with comparable type of meiosis. Failure to hatch and cytological changes in stages immediately following treatment have been the criteria of injury. Preliminary results were first published in 1938 (Whiting, 1938). Details of technique and hatchability effects, as well as extensive bibliography, are given elsewhere (Whiting, 1945); cytological effects and their correlation with mortality and dose are presented here in detail.

Dose-hatchability Relationships

Hatchability effects may be summarized briefly. No correction for control hatchability is made since it is so close to 100 per cent. Eggs x-rayed in diplotene and allowed to develop parthenogenetically give a dose-hatchability curve which appears to be linear at low doses and to become "mixed" at high doses; they have 50 per cent mortality at 12,000 *r* and 100 per cent at about 45,000 *r*; they showed no significant change in hatchability in preliminary and inadequate tests of time-intensity differences. Those treated in early diplotene (laid during the eleventh and twelfth hours after treatment) show no change in hatchability at any dose with fractionated treatment, those treated in late diplotene (laid during the ninth and tenth hours after treatment) show a significant increase at high doses with fractionated treatment. The dose-hatchability curve for combined diplotene owes much of its mixed character at high doses to late diplotene, early diplotene response being more nearly linear.

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Eggs x-rayed in late metaphase I and allowed to develop parthenogenetically show a linear decline in hatchability with increasing dose and have 50 per cent mortality at 375 *r*, 100 per cent at about 1,400 *r*; they show no change in dose-hatchability relationships with aging between treatment and oviposition, time-intensity differences or fractionation of dose.

When Habrobracon females are mated, about two-thirds of the eggs are fertilized. If treated females are mated to untreated males, the survival of any appreciable number of eggs through the aid of normal spermatozoa would increase percentage of hatchability thereby indicating the presence of recessive lethal effects by comparison with hatchability of eggs from treated unmated females. Table I demonstrates that most, if not all, of the lethal effects induced in these stages by x-rays are dominant, at least in respect to hatchability. This is rather surprising at first glance but in treated metaphase I, as pointed out below, chromosomal deletions appear to be relatively large and in either stage, it is possible that deletions small enough to act as recessives in fertilized eggs may not kill the individual until after hatching in unfertilized eggs. Lethals which are recessive in diploids may be due to such minute losses as to exert their effects only after hatching in haploids. Perhaps viable deficiency heterozygotes are so rare that hatchability of irradiated eggs is not perceptibly altered by fertilization with untreated spermatozoa. In any case, conditions are well suited to an analysis of dominant lethal ratios induced by x-rays in identifiable stages of meiosis and, although the chromosomes present

TABLE I

Hatchability percentages for eggs of treated females, unmated and mated to untreated males

Stage treated	Dose in *r* units	Unmated females		Mated females	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
Metaphase I	560	319	39.8±2.7	318	40.5±2.7
Prophase I	5,600	137	71.5±2.6	126	70.6±4.0
	22,400	100	19.0±3.9	182	19.2±2.9
Controls	0	127	98.4±1.1	363	98.6±0.6

difficulties, the eggs themselves are easily handled, fixed and stained for observation.

About 40,000 eggs were collected and observed for hatchability. Records were kept of the results from individual females in all cases so that aberrant behavior in eggs from any individual could be recognized. Such behavior was extremely rare.

From the work of Sax (1938, 1940), Fabergé (1940) and others on dose-chromosome injury curves, certain tentative conclusions were drawn concerning cytological effects before study of chromosomes was begun. For diplotene it was assumed that the great majority of chromosome breaks must undergo restitution; that broken ends of chromosomes within the same cell increase as dose increases, permitting complicated reunions (translocations, large interstitial deletions) so that lethal individual chromosome changes tend to be due increasingly to more than one ionization, especially in late diplotene; that bridges can be formed in either meiotic division or in both, due to lateral fusion of the broken ends of chromatids whenever two adjoining chromatids are broken by a single ionization. From the work of Sturtevant and Beadle (1936) and of McClintock (1941) it was thought that bridges in division I might be permanent or delayed in breakage and might offer an explanation for some, at least, of the high resistance of this stage to irradiation.

Concerning metaphase I it was assumed, because of the linear relationship of hatchability to dose, that injuries were in the form of terminal deletions or of minute interstitial deletions, in other words, injuries due to single ionizations. The high sensitivity of this stage suggested that most injuries must be permanent. It was doubted that a single ionization would break two chromatids due to the degree of separation in late metaphase I and so the occurrence of bridges in either division from this cause seemed improbable. There was also the possibility that high metaphase sensitivity might be due to "physiological" effects, stickiness, etc., which would result in fusion bridges, delay in division or death.

Cytological Observations

Cytology of controls. The cytology of the stages before metaphase I has not been studied in detail, either in control or irradiated material, because of the small size and large numbers of chromosomes and of their elongate and diffuse condition. Synapsis occurs in very young oocytes and the subsequent behavior through condensation appears to have nothing exceptional about it. Changes take place slowly and are not obvious in character until just before condensation of chromosomes (diakinesis) when tetrads move to periphery of the nucleus. Most students of hymenopteran cytology would question the conclusion that the stored oocyte is in an orthodox and identifiable stage, late metaphase I. They state that the chromatin has reverted to a resting stage or has formed an abortive spindle, a compact clump or a composite body, etc. Speicher (1936) finds that the most advanced eggs in the Habrobracon egg sac are in "early anaphase of the first maturation" which the author prefers to call late metaphase. Speicher's observations that distinct chromosomes are present, are in the form of tetrads (Fig. 1) and are ten in number has been repeatedly checked by the author and cannot be questioned. They show the forms expected for tetrads and each resolves immediately into two pairs of dyads upon completing division I. The conclusion must be drawn either that Habrobracon differs from many other Hymenoptera in having orthodox oogenesis or that its chromosomes retain more easily their individuality when fixed.

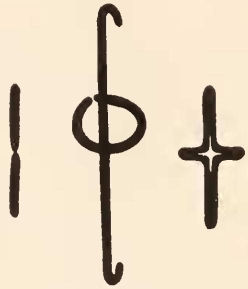

FIGURE 1. Three tetrads from one late metaphase I spindle. Untreated. Drawn from whole mount of egg with aid of a camera lucida. Semi-diagrammatic. × 4,625.

The stages of normal oogenesis following oviposition, as described by Speicher, are briefly as follows. During the process of oviposition the maturation spindle is moved from dorsal to ventral side of the egg. It then passes into telophase I. The second division follows immediately. The four groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (functional nucleus) sinks deeper into the egg, a membrane forming as it moves. Nucleus 1a soon disintegrates, 1b and 2a unite and form a metaphase plate which divides and then disintegrates. Cleavage is of the usual insect type, with nuclei moving about until blastoderm formation when cell membranes first appear. The stages following oviposition are the ones which were studied after irradiation.

No evidences of displaced chromosomes or of aberrant conditions resembling those observed in irradiated eggs were found by Speicher or by the author in large numbers of control eggs studied.

Cytology of irradiated eggs. In experiments concerned with cytological effects, eggs from control and treated females were incubated according to standard schedules, dropped into fixative (formalin-acetic-alcohol), punctured at the posterior end to facilitate fixation, treated with the Feulgen technique and mounted whole in balsam. Control hatchability tests were made of eggs treated at the same time as those fixed. Slides were made of about 2,500 eggs.

After treatment in diplotene acentric fragments, dicentrics or both may occur in division I (Fig. 2a, b, c) or in division II or in both divisions. Bridges in division I may be permanent and can be seen bulging at the side when nucleus 1b moves towards 2a, indicating that chromatin bridges are tensile but not elastic (Fig. 2c). Acentric fragments remain visible throughout both divisions. No evidences of stickiness or of clumping of chromatin (Fig. 2a, b, c) or of retardation of meiosis are apparent for doses up to lethal (45,000 r). Of eggs treated in diplotene with 44,800 r, 1.1 per cent died at first cleavage, 30.4 per cent with a few nuclei, 54.3 per cent with many nuclei and 14.2 per cent at blastoderm.

Immediately after irradiation in late metaphase I (Fig. 1), chromosomes show no apparent change but at telophase I acentric fragments are left within the spindle and these remain visible throughout division II (Fig. 2d, e). They are often almost as large as entire chromosomes and can usually be identified as double structures. No bridges have been seen in division I in over 1,500 eggs observed. In division II bridges occur and after heavy treatment (2,000 *r*) several may be seen in each second division spindle (Fig. 2e). Small fragments occasionally appear in division II spindles. No evidences of stickiness or of clumping (Fig. 2d, e) or of retardation of meiosis occur in development following treatment with lethal dose of this stage which, except for absence of bridges in division I, behaves cytologically as treated diplotene. Percentages of eggs with fragments in division I and mean number of fragments per treated increase linearly with increased dose (Whiting, 1945). All eggs exposed in late metaphase I to 2,016 *r* undergo some development. 2.4 per cent die in first cleavage, 7.2 per cent with a few nuclei, 71.4 per cent with many nuclei and 19.0 per cent at blastoderm stage. In spite of their high sensitivity, some eggs treated in metaphase I developed to the fifth cleavage (expected) after 15,000 *r*, to metaphase II after 25,000 *r*, to pronucleus after 35,000 *r* and one to anaphase II after 200,000 *r*. No records were kept of rate of development at these higher doses.

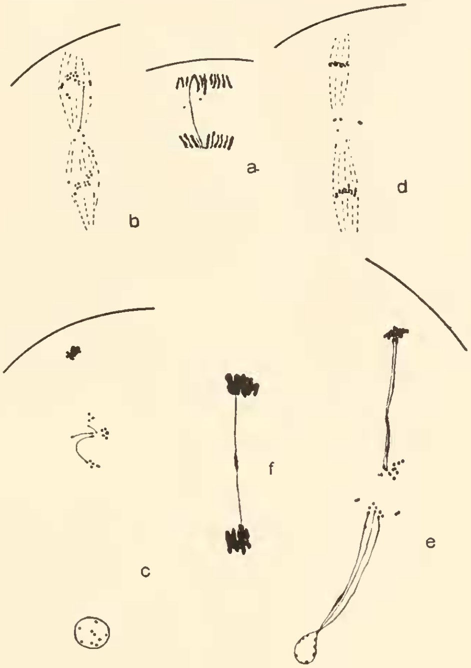

FIGURE 2. Illustrations were drawn from whole mounts of eggs with the aid of a camera lucida. *a, b,* and *c,* eggs were irradiated in late diplotene with 44,800 *r*; *a,* telophase I. × 1,500, *b,* metaphase II. × 875; *c,* telophase II. × 1,250; *d, e,* and *f,* eggs were irradiated in late metaphase I with 2,000 *r*; *d,* metaphase II. × 875; *e,* telophase II. × 1,250; *f,* third cleavage, telophase. × 4,550.

The similar patterns of stage at death for both diplotene and metaphase I at their respective lethal doses indicate that, in spite of the great difference in sensitivity between the stages, cause of death is of the same nature in both. These data on time of death check what has often been noted, especially in respect to mature spermatozoa, that so-called lethal doses are not actually lethal to the treated cell itself but, instead, to its descendents. The fact that the oocyte continues to function normally and that death does not occur until it becomes an embryo, supports the argument that cytoplasmic injury is not at the basis of mortality. It is due, rather, to loss of parts of chromosomes during meiosis following irradiation and to resulting incomplete chromosome complements in every cleavage nucleus.

Bridges occur in cleavage I after treatment in either stage indicating that, if chromatids are already split when treated, there occurs lateral fusion of broken ends of half-chromatids. If they are not split when treated the split occurring in the first mitosis must have been incomplete in the broken chromatid or have resulted in lateral fusion of broken ends of daughter chromosomes. Bridges appear in subsequent cleavages. Fragments, which also occur in cleavage, could not be explained at first until it was noted that they are tapering at the ends and that they result from double breaks in a bridge which releases a thickened middle portion (Fig. 2f). Fragments were not observed after every mitosis in the same embryo although bridges, if present, appear in all cleavage figures.

Correlation of Injury with Chromosome Form when Treated

It is perhaps unwise to devote much time and space to the subject of the correlation of the nature of the injuries and the form of the chromosomes when treated in view of the small size of Habrobracon chromosomes and the disagreement of investigators in this field. Obviously, there is a correlation. The studies of Sax (1938, 1940), Fabergé (1940) and McClintock (1938) will be used as a basis of a brief discussion, since the results of these investigations are consistent with their theories.

Chromosome injuries fall into two classes, those caused by single ionizations and those caused by more than one. The former consist of terminal deletions and minute interstitial deletions. Two identical terminal deletions can be induced by a single ionization if two chromatids are sufficiently close together. When this happens, lateral fusion of broken ends occurs resulting in a dicentric, from parts of the two chromatids still attached to spindle fibers, and an acentric, from the released and fused ends. Single terminal deletions can be induced by single ionizations and this appears to be the rule when chromatids are widely separated. An acentric is ultimately lost and a dicentric forms a bridge when its two spindle fiber attachment points (centromeres) are pulled apart. If the bridge does not break, an entire chromosome may be missing from a daughter cell. If it does break, the resulting chromosomes are incomplete and each daughter cell will have an incomplete chromosome and, therefore, an incomplete set of genes. Such a terminally incomplete chromosome may continue to form a bridge in each subsequent division, either by failing to split completely or by a lateral fusion of the broken ends after splitting. This appears to be the general rule but McClintock (1941) has found that when such an incomplete chromosome occurs in the sporophyte tissue of maize, it forms no bridge.

An interstitial deletion caused by a single ionization in a chromosome would mean the loss of genes and would be lethal if they were numerous or of sufficient importance but it would not be cytologically apparent in subsequent divisions because of its small size.

Injuries which must be due to more than one ionization since they involve breaks in chromosomes too far apart to be caused by a single ionization are large inversions, large interstitial deletions and translocations. Inversions would not be apparent, either cytologically or in effect on viability of the embryo receiving them in the present study since they would be induced after synapsis and crossing over and the inversion of a block of genes would probably have no lethal effect. Large interstitial deletions would have a lethal effect but could not be identified in material used in these experiments. Translocations might be lethal and would be visible as bridges should centric parts of non-homologues become attached to each other. Such bridges cannot be distinguished cytologically from those resulting from double terminal deletions in this material.

The nature of the hatchability curves suggests that most injuries in early diplotene and late metaphase I at all doses and in late diplotene at low doses are caused by single ionizations, that many injuries in late diplotene at high doses are caused by more than one ionization. Since there is no reason to suppose that the nature of original breaks would be changed by higher doses it is presumed that the number of single breaks per cell increases with high dose and allows greater opportunity for new combinations because of increased number of broken ends available at any one time. This would take for granted the breaking of single chromatids per ionization for if two were broken the lateral fusion of broken ends would prevent translocations, fusion with more distant chromosomes. The reduction in injury by fractionation of dose is explained on the grounds that, with repeated smaller doses, fewer free ends are available at any given time for new combinations and the intervals between treatments afford an opportunity for restitution or changes in broken ends to occur so that they are no longer capable of joining with other broken ends formed by later treatments.

Three conditions seem to be of importance, then, in determining response of the chromosomes here studied to irradiation. These are (1) relation of tetrads to each other in the nucleus, (2) degree of separation of adjoining chromatids within a tetrad and (3) nature and degree of tension on chromosomes. Each of the three stages will be discussed briefly from these points of view.

In early diplotene the tetrads are evenly distributed within the nucleus, sister chromatids are in contact, homologues separated except at chiasmata, and neither traction of the spindle fibers nor terminalization has begun. Most breaks will be temporary because of lack of tension and relaxed state of the chromosomes. Translocations should be possible but the majority of breaks will involve both sister chromatids with the production of acentrics and dicentrics. Permanent double breaks can occur either between centromeres and proximal chiasmata (with production of bridge in division II) or distal to chiasmata (producing bridge in division I if distal to "odd" chiasmata, in division II if distal to "even") since the slight tension which exists is equally exerted everywhere along the length of the chromosome.

In late diplotene the tetrads move peripherally but are still widely separated, terminalization (movement of chiasmata towards ends of tetrads) has begun, as well as movement of centromeres away from each other, and chromatids are not so closely associated, especially toward ends of chromosomes. Single and double breaks will occur (the latter nearer the centromere) and more of them will be permanent because of new tensions. Bridges should be less frequent in division I than in the case of early diplotene but this has not been checked. This stage will be somewhat more sensitive and will exert its lethal effects through translocations and large interstitial deletions as well as through double terminal deletions.

In late metaphase I, the tetrads are isolated from each other and stable in position on the spindle so that interchanges between them would not be expected. Centromeres are pulled far from each other and chiasmata resist further terminalization (Fig. 1) so that tension exerted between centromeres and proximal chiasmata is very great, tension exerted distal to chiasmata not so great. Ionizations will cause double breaks near centromeres where sister chromatids are closely approximated and these will all be permanent because of the extreme tension. They will result in large double fragments (acentrics) in division I, bridges (dicentrics) in division II. Breaks induced towards ends of chromosomes, and especially distal to chiasmata, will be less likely to be permanent and more likely to be single. There will be few or no bridges in division I and single fragments will appear in division I or division II (McClintock, 1938).

Any injury to a tetrad which results in a single bridge in division II reduces the chance of hatching of the egg by fifty per cent; in division I the effect is the same if the bridge breaks promptly. If it is delayed in breaking or does not break the hatchability of the egg is not affected, since an incomplete chromatid is thereby restrained from entering the ootid nucleus. A single terminal deletion reduces the chance of hatching by twenty-five per cent.

With ten tetrads of the diverse forms found in Habrobracon, combinations of changes induced by single ionizations can become very complex. If added to these are the complication of translocation and of large interstitial deletions (characteristic especially, perhaps, of late diplotene) the great resistance of diplotene is to be wondered at. The author (Whiting, 1945) has reviewed the data here reported in the light of the numerous theories devised to explain differential sensitivity of chromosomes to x-rays and has found that the only one which applies is that put forth by Goodspeed in 1929. He suggested tension as the important factor. It seems highly probable that numerous breaks do occur in the evenly distributed, diffuse, slowly moving chromosomes of diplotene but that the majority of them is temporary. Healing or restitution must take place quickly for there is always some movement and these chromosomes ultimately go through the same stresses as those treated in late metaphase I and, in addition, those attendent upon condensation and complete terminalization. Their response to fractionation also argues for relatively rapid restitution.

The development of individuals, normal in appearance and in reproductive activity and with normal descendents, after treatment in diplotene with 35,000 *r*, illustrates graphically the resistance of this stage to permanent injury by ionizations.

Sax (1942) summarizes the information available on "physiological" effects of x-rays, one of which is stickiness of chromatin. It has been found that condensed chromosomes are most sensitive in respect to stickiness, that such effects are temporary, delay subsequent division, have a threshold dose, are lethal only after very high doses and result in "fusion" bridges if the cell divides before recovery. The stage in the present study most likely to show the effects of stickiness in the form of fusion bridges is division I after treatment of metaphase I. This is the only division which shows bridges of no kind even after doses much higher than lethal. A delay of twenty-four hours between treatment and resumption of meiosis does not increase hatchability, meiosis is not appreciably delayed after irradiation, there is no threshold effect (down to 50 *r*). It should be emphasized again that, wide apart as are the lethal doses for diplotene and metaphase I, at their respective lethal doses, the pattern of stages at death is the same, the same percentage dies at first cleavage, at blastoderm, etc.; in other words, 45,000 *r* has no more drastic effect on development of treated diplotene than 1,400 *r* on metaphase I. All evidence indicates that cause of death is of the same order for both stages and that "physiological" effects are of every minor importance, and not appreciably different in the two stages.

Sturtevant and Beadle (1936) failed to recover an expected genetic type of chromosome aberration correlated with a dicentric in division I. They suggested that in a form like Drosophila where the four meiotic nuclei lie in a row and where a terminal one alone functions, a bridge in division I might fail to break, or might be delayed in breaking, thereby tying together injured chromatids and allowing uninjured ones to pass to the terminal nuclei. McClintock (1941) also offers as explanation for the failure to obtain expected genetic results correlated with bridges in division I of maize, the selective effect of these bridges on broken chromatids. Terminal nuclei (one of which becomes the functional megaspore) tend to receive the uninjured chromatids. Figure 3c demonstrates that bridges in division I in Habrobracon eggs do not break, at least in some cases.

In divisions following treatment of diplotene to which this selection of injured chromatids for elimination would apply, the chances of having bridges in the second division are as frequent as in the first or more so and selection through permanence of bridges would apply, therefore, only in the simplest kind of injury and that to but one or very few tetrads since any number of breaks would be certain to produce some bridges in division. This selection, although it undoubtedly occurs, cannot explain more than a small amount of resistance of diplotene. It would be expected to apply especially with low doses when but a single break occurs in a single tetrad.

The wide difference in size of lethal doses (45,000 *r*–1,400 *r*) of such closely related stages of the same cell, the unreduced Habrobracon egg, confirms the truth of the conclusion made long ago (1906) by Krause and Ziegler in an extensive and critical study of tissue injury by x-rays, that it is less the kind of cell than its stage at the time of treatment which determines sensitivity.

The facts and theories just presented are of interest in connection with a discussion of dominant lethals by Pontecorvo (1942). He explains dominant lethal effects in Drosophila spermatozoa by assuming that single chromosome breaks are produced by radiations at a rate proportional to radiation dose and that these neither undergo restitution nor participate with other breaks in the same nucleus in rearrangements. "Chromosomes with broken ends give rise to a cycle of breakage-fusion-bridge phenomena in development." He also writes, "It is therefore an open question whether sister unions are so frequent as to cause a considerable portion of dominant lethality. Should this be the case, the trend of the curve of dominant lethality could be explained. Most dominant lethality would be determined by single-break sister unions at low dosages and as the dosage increased lethal changes of the other two types (translocations and deletions) would come to play an increasing part." Translocations and deletions would not be produced actually until syngamy since breaks appear to remain open in the sperm chromosomes until that time.

Broken chromosome ends do undergo restitution or participate with other broken ends in the egg very soon after treatment but the final contribution to the zygote may be the same as that made by the irradiated sperm; viz., a chromosome with a broken end which will give rise to the breakage-fusion-bridge cycle in the first cleavage as well as in subsequent ones. Most dominant lethality in the present study is, without much doubt, caused by single ionizations and only in late diplotene at high doses does treatment appear to cause a high percentage of death from the cooperation of two or more ionizations.

It is of interest in this connection to note that the dose-injury curve for dominant lethality in the spermatozoa of Drosophila (Sonnenblick, 1940; Demerec and Fano, 1944) and for Habrobracon (Heidenthal, 1945) is of the same nature as that for late diplotene.

CONCLUSION

1. (Tentative) The majority of dominant lethals induced in late diplotene by low doses (to 11,000 *r*) and in early diplotene and in late metaphase I by all doses through lethal, in Habrobracon eggs, is caused by single ionizations which break adjoining chromatids. Lateral fusion of broken ends results, followed by continued breakage-fusion-bridge phenomena in cleavage. With doses above 11,000 *r* in late diplotene an increasing number of lethal changes arises from two or more ionizations (translocations, large interstitial deletions). 2. Lethal doses are not lethal to the treated cell (oocyte) itself but to its descendents (embryo). Fragmentation of chromosomes is not lethal, loss of fragments is. 3. The nature and degree of chromosome injury can be correlated with the form of the chromosome and with forces acting upon it during and immediately following treatment. 4. The kind of cell is less important than its stage in determining sensitivity to x-rays. 5. Tension is the main factor in determining permanence of breaks caused by ionizations, chromosome form and movement in determining the nature of the new combinations of broken ends. 6. The chromosome phenomena here dealt with are common ones in the majority of animals and plants and it is predicted that, when metaphase and anaphase are sufficiently studied in other forms, they will be found to be the stages most sensitive to x-rays. This has proved to be the case for Sciara (Metz and Bozeman, 1940; Reynolds, 1941) and for Trillium (Sparrow, 1944).

SUMMARY

Unlaid Habrobracon eggs x-rayed in diplotene (lethal dose about 45,000 *r*) and allowed to develop parthenogenetically, show fragments, bridges or both in division I; either or both in division II. Bridges in division I may be permanent.

Unlaid eggs x-rayed in late metaphase I (lethal dose about 1,400 *r*) show fragments but no bridges in division I; bridges, fragments or both in division II.

An explanation of difference in cytological effects of x-rays on these stages and of the differences between them in sensitivity and in nature of survival curves is attempted through comparison with studies on forms with larger chromosomes.

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